Surprisingly, I_{Ks} amplitude remained unchanged with over-expression of either KCNQ1 or KCNE1, suggesting a tightly regulated system for maintaining KCNQ1/KCNE1 surface density.

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A Previously Unrecognized Conductance is a Critical New Player in the Pacemaker of Cardiomyocytes Derived from Human Embryonic Stem Cells

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The timely appearance and proper functioning of pacemaker activity is a critical feature of heart physiology. Two main mechanisms have been proposed: (1) The ''voltage-clock'', where the hyperpolarization-activated funny current If causes diastolic depolarization that triggers action potential cycling; (2) The ''Ca2+ clock'', where cyclical release of Ca2+ from Ca2+ stores depolarizes the membrane during diastole via activation of the Na+-Ca2+ exchanger (NCX). However, these pacemaker mechanisms remain highly controversial. Here, we used human embryonic stem cell-derived cardiomyocytes (hESC-CMs) to study the embryonic pacemaker mechanisms of the human heart. Combined current- and voltage-clamp recording from the same hESC-CM and blocking If with zatebradine or ZD7288 and NCX with KB-R7943 or FRCRCF peptide revealed distinct pacemaker phenotypes. Results showed that the ''voltage clock'' and ''Ca2+ clock'' pacemakers can coexist in the same cell, but can also occur in a mutually exclusive fashion in other cell populations. Interestingly, all these pacemaker phenotypes shared a depolarizing drift of the maximal diastolic potential (MDP) following exposure of cells to blockers of the ''voltage'' and ''Ca2+ clocks'', suggesting that both mechanisms converge to a common pacemaking component. This MDP depolarization arises from inhibition of a previously unrecognized conductance in hESC-CMs. Remarkably, blockade of this conductance leads to depolarization of the MDP and suppresses pacemaker activity. Data are discussed on how this conductance plays a crucial role in human embryonic cardiac automaticity.

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Real-Time Human and Guinea Pig Action Potentials Anthropomorphized from Neonatal Mouse Cardiomyocytes

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The murine cardiac action potential waveform can be anthropomorphized into that of a human-like waveform in real time, through a novel dynamic-clamp method known as the cell-type transforming clamp (CTC). In the CTC, a computationally calculated virtual conductance is inserted into the cell in real time, to compensate for the differences between murine and human sarcolemmal currents. By so doing, the CTC anthropomorphizes the membrane potential without clamping it, thereby enabling the investigation of drug- or mutation-induced arrhythmogenic phenotypes in the appropriate human action potential context (but in the experimentally powerful mouse animal model).

We are using a real-time implementation of a genetic algorithm that optimizes the morphology of a theoretical model, in order to match the murine action potential recorded from a real cell. We present a comparison of human and guinea pig action potentials anthropomorphized in real time, from neonatal mouse cardiomyocytes.

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Estrogen Therapy Abolishes Spontaneous Ventricular Arrhythmias in Right Ventricular Failure Induced by Pulmonary Hypertension

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We previously have shown that pulmonary hypertension (PH)-induced right ventricular failure (RVF) is associated with increased incidence of sudden death caused by spontaneous ventricular fibrillation (VF). We also discovered that estrogen (E2) therapy rescues severe PH and RVF and results in 100% survival. Here we hypothesized that E2 abolishes spontaneous VF associated with RVF by restoring RVEF, reversing fibrosis and restoring PH-induced downregulation of repolarizing K-channel proteins Kv1.5 and KCNE-2 and SERCA-2a expression. Chronic PH-associated RVF was induced in male rats by s.c. monocrotaline (MCT, 60 mg/kg, n=10). Some MCT-rats were treated with E2 (42.5 ug/kg/day, s.c.) from day 21 (severe PH-stage) to day-30 (n=8). Saline treated rats served as control (n=8). At ~day 30, hearts were studied in isolated-perfused Langendorff setting. RV-epicardial activation pattern was optically mapped using fluorescent voltage-sensitive dye (RH-237). By ~day 30, 30% RVF rats died suddenly but none in the control or E2-groups. RVF hearts manifested EADs and spontaneous VF during normal Tyrode's perfusion with wavefront dynamics supported by both focal and multiple wavelet patterns. No VF was initiated in any of the control or E2-treated hearts. SERCA-2a was reduced (~15-fold) in RVF (0.06 ± 0.01 vs. 1 ± 0.26 control) that was reversed in E2-group (0.77 ± 0.13, $p<0.05$ vs. RVF). Kv1.5 was reduced ~8 fold in RVF (0.12 ± 0.03 vs. 1 ± 0.18 in control, $p<0.05$) and E2 partially restored Kv1.5 (0.46 ± 0.06, $p<0.05$). KCNE2, an ancillary K-channel subunit, was reduced ~3-fold in RVF (0.3 ± 0.1 vs. 1 ± 0.1, $p<0.05$) that was reversed by E2 (0.9 ± 0.05, $p<0.05$). RVF was associated with moderate RV-fibrosis and severe reduction in RV-ejection fraction (RVEF) from 65 ± 1 to $33 \pm 3\%$. E2- group did not show any fibrosis and RVEF was preserved ($60 \pm 2\%$). In conclusion, E2-therapy rescues RVF and prevents VF by reversing PH-induced RV-fibrosis and reduced repolarization-reserve.

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Anisotropic Biaxial Stretch Slows Longitudinal and Transverse Conduction in Micropatterned Mouse Ventricular Myocyte Cultures

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The effects of stretch on cardiac conduction velocity are controversial, and several counteracting mechanisms have been proposed. Conflicting reports of conduction velocity increase and decrease under cardiac loading have been reported. These changes have been attributed to stretch modulation of ion channels, cell-cell junctions, cell capacitance, and properties of the interstitium. To separate the effects of tissue geometrical changes from intrinsic changes in myocyte conduction and to eliminate effects of stretch on interstitial electrical properties, neonatal murine cardiomyocytes were cultured on micropatterned stretchable substrates for optical mapping of excitation conduction velocity. A homogeneous anisotropic biaxial strain field of 14% in the primary direction and 3.6% in the secondary direction was applied to these substrates, where the primary direction of stretch was oriented either parallel or perpendicular to the longitudinal axis of the aligned cell culture. When the primary direction of strain was oriented parallel to the longitudinal cell culture axis, the longitudinal conduction velocity slowed to 72% ± 3% and recovered to 95% ± 14% of baseline conduction following unloading, while the transverse conduction velocity slowed to 75% ± 3% and recovered to 137% ± 19% (n=3, mean ± SEM). When the primary direction of strain was oriented along the transverse cell culture axis, the transverse conduction velocity slowed to 77% ± 6%, while the longitudinal conduction velocity slowed to 84% ± 9%, neither recovering following unloading (n=3, mean ± SEM). Conduction velocity slowed in both longitudinal and transverse directions under biaxial strain and substantially recovered following unloading in the case of primarily axial stretch.

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A Novel Role for Ephaptic Coupling in Cardiac Conduction: An Experimental and Modeling Study

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Introduction: We demonstrated that edema slows conduction (θ and increases anisotropy (AR_θ). Existing mathematical models incorporating only gap junctional (Gj) coupling cannot explain these data. We hypothesized that inhibiting ion channels during edema would unmask ephaptic coupling effects.

Methods: θ and AR_θ were quantified by optical mapping in Langendorff-perfused guinea pig ventricles. Mannitol (26.1g/l) was perfused to increase VIS. INa, which partially colocalizes with Gj, was inhibited by flecainide (0.5μM). IK1, which exhibits low intercalated disk colocalization with Gj, was inhibited by $BaCl_2$ (10 μM). Conduction was mathematically modeled in a 2D tissue slab with both Gj and ephaptic coupling and appropriate INa and IK1 cellular distributions.

Results: During control, longitudinal (θ_L) and transverse (θ_T) θ were 51.9 ± 1.0cm/s and 21.2 ± 0.6cm/s respectively; AR_θ was 2.5 ± 0.1. Flecainide decreased both θ_L and θ_T by $17 \pm 2\%$ (n=5, $p<0.05$ vs. control). $BaCl_2$ increased both θ_L and θ_T by $23 \pm 2\%$ (n=4, $p<0.05$ vs. control). Neither altered AR_θ (p=ns vs. control). Mannitol decreased θ_L ($16 \pm 3\%$, n=3, $p<0.05$) and θ_T ($26 \pm 2\%$, n=3, $p<0.05$) and increased AR_θ to 3.0 ± 0.1